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(54) Staphylococcus aureus polynucleotides and sequences

(57) The present invention provides polynucleotide sequences of the genome of *Staphylococcus aureus*, polypeptide sequences encoded by the polynucleotide sequences, corresponding polynucleotides and polypeptides, vectors and hosts comprising the polynucleotides, and assays and other uses thereof. The present invention further provides polynucleotide and polypeptide sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use.

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children and adolescents more than adults and it is associated with non-penetrating injuries to bones. Infection typically occurs in the long end of growing bone, hence its occurrence in physically immature populations. Most often, infection is localized in the vicinity of sprouting capillary loops adjacent to epiphysial growth plates in the end of long, growing bones.

Skin infections

S. aureus is the most common pathogen of such minor skin infections as abscesses and boils. Such infections often are resolved by normal host response mechanisms, but they also can develop into severe internal infections. Recurrent infections of the nasal passages plague nasal carriers of *S. aureus*.

Surgical Wound Infections

Surgical wounds often penetrate far into the body. Infection of such wound thus poses a grave risk to the patient. S. aureus is the most important causative agent of infections in surgical wounds. *S. aureus* is unusually adept at invading surgical wounds; sutured wounds can be infected by far fewer *S. aureus* cells then are necessary to cause infection in normal skin. Invasion of surgical wound can lead to severe *S. aureus* septicaemia. Invasion of the blood stream by *S. aureus* can lead to seeding and infection of internal organs, particularly heart valves and bone, causing systemic diseases, such as endocarditis and osteomyelitis.

Scalded Skin Syndrome

S. aureus is responsible for "scalded skin syndrome" (also called toxic epidermal necrosis, Ritter's disease and Lyell's disease). This diseases occurs in older children, typically in outbreaks caused by flowering of *S. aureus* strains produce exfoliation(also called scalded skin syndrome toxin). Although the bacteria initially may infect only a minor lesion, the toxin destroys intercellular connections, spreads epidermal layers and allows the infection to penetrate the outer layer of the skin, producing the desquamation that typifies the diseases. Shedding of the outer layer of skin generally reveals normal skin below, but fluid lost in the process can produce severe injury in young children if it is not treated properly.

Toxic Shock Syndrome

Toxic shock syndrome is caused by strains of *S. aureus* that produce the so-called toxic shock syndrome toxin. The disease can be caused by *S. aureus* infection at any site, but it is too often erroneously viewed exclusively as a disease solely of women who use tampons. The disease involves toxaemia and septicaemia, and can be fatal.

Nocosomial Infections

In the 1984 National Nocosomial Infection Surveillance Study ("NNIS") *S. aureus* was the most prevalent agent of surgical wound infections in many hospital services, including medicine, surgery, obstetrics, pediatrics and newborns.

Resistance to drugs of S. aureus strains

Prior to the introduction of penicillin the prognosis for patients seriously infected with *S. aureus* was unfavorable. Following the introduction of penicillin in the early 1940s even the worst *S. aureus* infections generally could be treated successfully. The emergence of penicillin-resistant strains of *S. aureus* did not take long, however. Most strains of *S. aureus* encountered in hospital infections today do not respond to penicillin; although, fortunately, this is not the case for *S. aureus* encountered in community infections.

It is well known now that penicillin-resistant strains of *S. aureus* produce a lactamase which converts penicillin to pencillinoic acid, and thereby destroys antibiotic activity. Furthermore, the lactamase gene often is propagated episomally, typically on a plasmid, and often is only one of several genes on an episomal element that, together, confer multidrug resistance.

Methicillins, introduced in the 1960s, largely overcame the problem of penicillin resistance in *S. aureus*. These compounds conserve the portions of penicillin responsible for antibiotic activity and modify or alter other portions that make penicillin a good substrate for inactivating lactamases. However, methicillin resistance has emerged in *S. aureus*, along with resistance to many other antibiotics effective against this organism, including aminoglycosides, tetracycline, chloramphenicol, macrolides and lincosamides. In fact, methicillin-resistant strains of *S. aureus* generally are multiply drug resistant.

information herein described stored in a data storage means. Such systems are designed to identify commercially important fragments of the *Staphylococcus aureus* genome.

Another embodiment of the present invention is directed to fragments, preferably isolated fragments, of the *Staphylococcus aureus* genome having particular structural or functional attributes. Such fragments of the *Staphylococcus aureus* genome of the present invention include, but are not limited to, fragments which encode peptides, hereinafter referred to as open reading frames or ORFs," fragments which modulate the expression of an operably linked ORF, hereinafter referred to as expression modulating fragments or EMFs," and fragments which can be used to diagnose the presence of *Staphylococcus aureus* in a sample, hereinafter referred to as diagnostic fragments or "DFs."

Each of the ORFs in fragments of the *Staphylococcus aureus* genome disclosed in Tables 1-3, and the EMFs found 5' to the ORFs, can be used in numerous ways as polynucleotide reagents. For instance, the sequences can be used as diagnostic probes or amplification primers for detecting or determining the presence of a specific microbe in a sample, to selectively control gene expression in a host and in the production of polypeptides, such as polypeptides encoded by ORFs of the present invention, particular those polypeptides that have a pharmacological activity.

The present invention further includes recombinant constructs comprising one or more fragments of the *Staphylococcus aureus* genome of the present invention. The recombinant constructs of the present invention comprise vectors, such as a plasmid or viral vector, into which a fragment of the *Staphylococcus aureus* has been inserted.

The present invention further provides host cells containing any of the isolated fragments of the *Staphylococcus aureus* genome of the present invention. The host cells can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic cell, such as a yeast cell, or a procaryotic cell such as a bacterial cell.

The present invention is further directed to polypeptides and proteins, preferably isolated polypeptides and proteins, encoded by ORFs of the present invention. A variety of methods, well known to those of skill in the art, routinely may be utilized to obtain any of the polypeptides and proteins of the present invention. For instance, polypeptides and proteins of the present invention having relatively short, simple amino acid sequences readily can be synthesized using commercially available automated peptide synthesizers. Polypeptides and proteins of the present invention also may be purified from bacterial cells which naturally produce the protein. Yet another alternative is to purify polypeptide and proteins of the present invention can from cells which have been altered to express them.

The invention further provides polypeptides, preferably isolated polypeptides, comprising *Staphylococcus aureus* epitopes and vaccine compositions comprising such polypeptides. Also provided are methods for vacciniating an individual against *Staphylococcus aureus* infection.

The invention further provides methods of obtaining homologs of the fragments of the *Staphylococcus aureus* genome of the present invention and homologs of the proteins encoded by the ORFs of the present invention. Specifically, by using the nucleotide and amino acid sequences disclosed herein as a probe or as primers, and techniques such as PCR cloning and colony/plaque hybridization, one skilled in the art can obtain homologs.

The invention further provides antibodies which selectively bind polypeptides and proteins of the present invention. Such antibodies include both monoclonal and polyclonal antibodies.

The invention further provides hybridomas which produce the above-described antibodies. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

The present invention further provides methods of identifying test samples derived from cells which express one of the ORFs of the present invention, or a homolog thereof. Such methods comprise incubating a test sample with one or more of the antibodies of the present invention, or one or more of the Dfs or antigens of the present invention, under conditions which allow a skilled artisan to determine if the sample contains the ORF or product produced therefrom.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the above-described assays.

Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the antibodies, antigens, or one of the DFs of the present invention: and (b) one or more other containers comprising one or more of the following:wash reagents, reagents capable of detecting presence of bound antibodies, antigens or hybridized DFs.

Using the isolated proteins of the present invention, the present invention further provides methods of obtaining and identifying agents capable of binding to a polypeptide or protein encoded by one of the ORFs of the present invention. Specifically, such agents include, as further described below, antibodies, peptides, carbohydrates, pharmaceutical agents and the like. Such methods comprise steps of: (a)contacting an agent with an isolated protein encoded by one of the ORFs of the present invention; and (b)determining whether the agent binds to said protein.

The present genomic sequences of *Staphylococcus aureus* will be of great value to all laboratories working with this organism and for a variety of commercial purposes. Many fragments of the *Staphylococcus aureus* genome will be immediately identified by similarity searches against GenBank or protein databases and will be of immediate value to *Staphylococcus aureus* researchers and for immediate commercial value for the production of proteins or to control gene expression.

The methodology and technology for elucidating extensive genomic sequences of bacterial and other genomes

libraries and for sequencing are provided below, for instance. A wide variety of *Staphylococcus aureus* strains that can be used to prepare *S aureus* genomic DNA for cloning and for obtaining polynucleotides of the present invention are available to the public from recognized depository institutions, such as the American Type Culture Collection (ATCC®).

The nucleotide sequences of the genomes from different strains of *Staphylococcus aureus* differ somewhat. However, the nucleotide sequences of the genomes of all *Staphylococcus aureus* strains will be at least 95% identical, in corresponding part, to the nucleotide sequences provided in SEQ ID NOS:1-5,191. Nearly all will be at least 99% identical and the great majority will be 99.9% identical.

Thus, the present invention further provides nucleotide sequences which are at least 95%, preferably 99% and most preferably 99.9% identical to the nucleotide sequences of SEQ ID NOS:1-5,191, in a form which can be readily used, analyzed and interpreted by the skilled artisan.

Methods for determining whether a nucleotide sequence is at least 95%, at least 99% or at least 99.9% identical to the nucleotide sequences of SEQ ID NOS:1-5,191 are routine and readily available to the skilled artisan. For example, the well known fasta algorithm described in Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85: 2444 (1988) can be used to generate the percent identity of nucleotide sequences. The BLASTN program also can be used to generate an identity score of polynucleotides compared to one another.

COMPUTER RELATED EMBODIMENTS

The nucleotide sequences provided in SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95%, preferably at least 99% and most preferably at least 99.9% identical to a polynucleotide sequence of SEQ ID NOS:1-5,191 may be "provided" in a variety of mediums to facilitate use thereof. As used herein, Òprovided" refers to a manufacture, other than an isolated nucleic acid molecule, which contains a nucleotide sequence of the present invention; *i.e.*, a nucleotide sequence provided in SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95%, preferably at least 99% and most preferably at least 99.9% identical to a polynucleotide of SEQ ID NOS:1-5,191. Such a manufacture provides a large portion of the *Staphylococcus aureus* genome and parts thereof (*e.g.*, a *Staphylococcus aureus* open reading frame (ORF)) in a form which allows a skilled artisan to examine the manufacture using means not directly applicable to examining the *Staphylococcus aureus* genome or a subset thereof as it exists in nature or in purified form.

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium which can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD- ROM; electrical storage media such as RAM and ROM; and hybrids of these categories, such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide sequence of the present invention. Likewise, it will be clear to those of skill how additional computer readable media that may be developed also can be used to create analogous manufactures having recorded thereon a nucleotide sequence of the present invention.

As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently know methods for recording information on computer readable medium to generate manufactures comprising the nucleotide sequence information of the present invention.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially- available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of data-processor structuring formats (*e.g.*, text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. Thus, by providing in computer readable form the nucleotide sequences of SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95%, preferably at least 99% and most preferably at least 99.9% identical to a sequence of SEQ ID NOS:1-5,191 the present invention enables the skilled artisan routinely to access the provided sequence information for a wide variety of purposes.

The examples which follow demonstrate how software which implements the BLAST (Altschul *et al.*, J. Mol. Biol. 215:403410 (1990)) and BLAZE (Brutlag *et al.*, Comp. Chem. 17:203-207 (1993)) search algorithms on a Sybase system was used to identify open reading frames (ORFs) within the *Staphylococcus aureus* genome which contain homology to ORFs or proteins from both *Staphylococcus aureus* and from other organisms. Among the ORFs discussed

accessing and processing the genomic sequence (such as search tools, comparing tools, *etc.*) reside in main memory 108, in accordance with the requirements and operating parameters of the operating system, the hardware system and the software program or programs.

BIOCHEMICAL EMBODIMENTS

Other embodiments of the present invention are directed to fragments of the *Staphylococcus aureus* genome, preferably to isolated fragments. The fragments of the *Staphylococcus aureus* genome of the present invention include, but are not limited to fragments which encode peptides, hereinafter open reading frames (ORFs), fragments which modulate the expression of an operably linked ORF, hereinafter expression modulating fragments (EMFs) and fragments which can be used to diagnose the presence of *Staphylococcus aureus* in a sample, hereinafter diagnostic fragments (DFs).

As used herein, an "isolated nucleic acid molecule" or an "isolated fragment of the *Staphylococcus aureus* genome" refers to a nucleic acid molecule possessing a specific nucleotide sequence which has been subjected to purification means to reduce, from the composition, the number of compounds which are normally associated with the composition. Particularly, the term refers to the nucleic acid molecules having the sequences set out in SEQ ID NOS:1-5,191, to representative fragments thereof as described above, to polynucleotides at least 95%, preferably at least 99% and especially preferably at least 99.9% identical in sequence thereto, also as set out above.

A variety of purification means can be used to generated the isolated fragments of the present invention. These include, but are not limited to methods which separate constituents of a solution based on charge, solubility, or size.

In one embodiment, *Staphylococcus aureus* DNA can be mechanically sheared to produce fragments of 15-20 kb in length. These fragments can then be used to generate an *Staphylococcus aureus* library by inserting them into lambda clones as described in the Examples below. Primers flanking, for example, an ORF, such as those enumerated in Tables 1-3 can then be generated using nucleotide sequence information provided in SEQ ID NOS: 1-5,191. Well known and routine techniques of PCR cloning then can be used to isolate the ORF from the lambda DNA library of Staphylococcus aureus genomic DNA. Thus, given the availability of SEQ ID NOS:1-5,191, the information in Tables 1, 2 and 3, and the information that may be obtained readily by analysis of the sequences of SEQ ID NOS:1-5,191 using methods set out above, those of skill will be enabled by the present disclosure to isolate any ORF-containing or other nucleic acid fragment of the present invention.

The isolated nucleic acid molecules of the present invention include, but are not limited to single stranded and double stranded DNA, and single stranded RNA.

As used herein, an "open reading frame," ORF, means a series of triplets coding for amino acids without any termination codons and is a sequence translatable into protein.

Tables 1, 2 and 3 list ORFs in the *Staphylococcus aureus* genomic contigs of the present invention that were identified as putative coding regions by the GeneMark software using organism-specific second-order Markov probability transition matrices. It will be appreciated that other criteria can be used, in accordance with well known analytical methods, such as those discussed herein, to generate more inclusive, more restrictive or more selective lists.

Table 1 sets out ORFs in the *Staphylococcus aureus* contigs of the present invention that are at least 80 amino acids long and over a continuous region of at least 50 bases which are 95% or more identical (by BLAST analysis) to an *S. aureus* nucleotide sequence available through Genbank in November 1996.

Table 2 sets out ORFs in the *Staphylococcus aureus* contigs of the present invention that are not in Table 1 and match, with a BLASTP probability score of 0.01 or less, a polypeptide sequence available through Genbank by September 1996.

Table 3 sets out ORFs in the *Staphylococcus aureus* contigs of the present invention that do not match significantly, by BLASTP analysis, a polypeptide sequence available through Genbank by September 1996.

In each table, the first and second columns identify the ORF by, respectively, contig number and ORF number within the contig; the third column indicates the reading frame, taking the first 5' nucleotide of the contig as the start of the +1 frame; the fourth column indicates the first nucleotide of the ORF, counting from the 5' end of the contig strand; and the fifth column indicates the length of each ORF in nucleotides.

In Tables 1 and 2, column six, lists the Reference" for the closest matching sequence available through Genbank. These reference numbers are the databases entry numbers commonly used by those of skill in the art, who will be familiar with their denominators. Descriptions of the numenclature are available from the National Center for Biotechnology Information. Column seven in Tables 1 and 2 provides the gene name" of the matching sequence; column eight provides the BLAST identity" score from the comparison of the ORF and the homologous gene; and column nine indicates the length in nucleotides of the highest scoring segment pair" identified by the BLAST identity analysis.

In Table 3, the last column, column six, indicates the length of each ORF in amino acid residues.

The concepts of percent identity and percent similarity of two polypeptide sequences is well understood in the art. For example, two polypeptides 10 amino acids in length which differ at three amino acid positions (*e.g.*, at positions

phylococcus aureus, particularly those that distinguish medically important strain, such as drug-resistant strains.

In addition, the fragments of the present invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on the binding of a polynucleotide sequence to DNA or RNA. Triple helix- formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Information from the sequences of the present invention can be used to design antisense and triple helix-forming oligonucleotides. Polynucleotides suitable for use in these methods are usually 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription, for triple-helix formation, or to the mRNA itself, for antisense inhibition. Both techniques have been demonstrated to be effective in model systems, and the requisite techniques are well known and involve routine procedures. Triple helix techniques are discussed in, for example, Lee *et al., Nucl. Acids Res.* 6: 3073 (1979); Cooney *et al., Science* 241: 456 (1988); and Dervan *et al., Science* 251: 1360 (1991). Antisense techniques in general are discussed in, for instance, Okano, *J. Neurochem.* 56: 560 (1991) and OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION, CRC Press, Boca Raton, FL (1988)).

The present invention further provides recombinant constructs comprising one or more fragments of the *Staphylococcus aureus* genomic fragments and contigs of the present invention. Certain preferred recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a fragment of the *Staphylococcus aureus* genome has been inserted, in a forward or reverse orientation. In the case of a vector comprising one of the ORFs of the present invention, the vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the ORF. For vectors comprising the EMFs of the present invention, the vector may further comprise a marker sequence or heterologous ORF operably linked to the EMF.

Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Useful bacterial vectors include phagescript, PsiX174, pBluescript SK and KS (+ and -), pNH8a, pNH16a, pNH18a, pNH46a (available from Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (available from Pharmacia). Useful eukaryotic vectors include pWLneo, pSV2cat, pOG44, pXT1, pSG (available from Stratagene) pSVK3, pBPV, pMSG, pSVL (available from Pharmacia).

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein- I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

The present invention further provides host cells containing any one of the isolated fragments of the *Staphylococcus aureus* genomic fragments and contigs of the present invention, wherein the fragment has been introduced into the host cell using known methods. The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or a procaryotic cell, such as a bacterial cell.

A polynucleotide of the present invention, such as a recombinant construct comprising an ORF of the present invention, may be introduced into the host by a variety of well established techniques that are standard in the art, such as calcium phosphate transfection, DEAE, dextran mediated transfection and electroporation, which are described in, for instance, Davis, L. *et al.,* BASIC METHODS IN MOLECULAR BIOLOGY (1986).

A host cell containing one of the fragments of the *Staphylococcus aureus* genomic fragments and contigs of the present invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF) or can be used to produce a heterologous protein under the control of the EMF.

The present invention further provides isolated polypeptides encoded by the nucleic acid fragments of the present invention or by degenerate variants of the nucleic acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (*e.g.,* an ORF) by nucleotide sequence but, due to the degeneracy of the Genetic Code, encode an identical polypeptide sequence.

Preferred nucleic acid fragments of the present invention are the ORFs depicted in Tables 2 and 3 which encode proteins.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. This is particularly useful in producing small peptides and fragments of larger polypeptides. Such short fragments as may be obtained most readily by synthesis are useful, for example, in generating antibodies against the native polypeptide, as discussed further below.

In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily employ well-known methods for isolating polpeptides and proteins to isolate and purify polypeptides or proteins of the present invention produced naturally by a bacterial strain, or by other methods. Methods for isolation and purification that can be employed in this regard include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immu-

may, also be employed as a matter of choice.

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (available form Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM 1 (available from Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter, where it is inducible, is derepressed or induced by appropriate means (*e.g.*, temperature shift or chemical induction) and cells are cultured for an additional period to provide for expression of the induced gene product. Thereafter cells are typically harvested, generally by centrifugation, disrupted to release expressed protein, generally by physical or chemical means, and the resulting crude extract is retained for further purification.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described in Gluzman, *Cell* 23: 175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines.

Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

Recombinant polypeptides and proteins produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

An additional aspect of the invention includes Staphylococcus aureus polypeptides which are useful as immunodiagnostic antigens and/or immunoprotective vaccines, collectively "immunologically useful polypeptides". Such immunologically useful polypeptides may be selected from the ORFs disclosed herein based on techniques well known in the art and described elsewhere herein. The inventors have used the following criteria to select several immunologically useful polypeptides:

As is known in the art, an amino terminal type I signal sequence directs a nascent protein across the plasma and outer membranes to the exterior of the bacterial cell. Such outermembrane polypeptides are expected to be immunologically useful. According to Izard, J. W. et al., Mol. Microbiol. 13, 765-773; (1994), polypeptides containing type I signal sequences contain the following physical attributes: The length of the type I signal sequence is approximately 15 to 25 primarily hydrophobic amino acid residues with a net positive charge in the extreme amino terminus; the central region of the signal sequence must adopt an alpha-helical conformation in a hydrophobic environment; and the region surrounding the actual site of cleavage is ideally six residues long, with small side-chain amino acids in the -1 and -3 positions.

Also known in the art is the type IV signal sequence which is an example of the several types of functional signal sequences which exist in addition to the type I signal sequence detailed above. Although functionally related, the type IV signal sequence possesses a unique set of biochemical and physical attributes (Strom, M. S. and Lory, S., J. Bacteriol. 174, 7345-7351; 1992)). These are typically six to eight amino acids with a net basic charge followed by an additional sixteen to thirty primarily hydrophobic residues. The cleavage site of a type IV signal sequence is typically after the initial six to eight amino acids at the extreme amino terminus. In addition, all type IV signal sequences contain a phenylalanine residue at the +1 site relative to the cleavage site.

Studies of the cleavage sites of twenty-six bacterial lipoprotein precursors has allowed the definition of a consensus amino acid sequence for lipoprotein cleavage. Nearly three-fourths of the bacterial lipoprotein precursors examined contained the sequence L-(A,S)-(G,A)-C at positions -3 to +1, relative to the point of cleavage (Hayashi, S. and Wu, H. C. Lipoproteins in bacteria. J Bioenerg. Biomembr. 22, 451-471; 1990).

It well known that most anchored proteins found on the surface of gram-positive bacteria possess a highly conserved carboxy terminal sequence. More than fifty such proteins from organisms such as *S. pyogenes, S. mutans, E. faecalis, S. pneumoniae,* and others, have been identified based on their extracellular location and carboxy terminal amino acid sequence (Fischetti, V. A. Gram-positive commensal bacteria deliver antigens to elicit mucosal and systemic immunity. ASM News 62, 405410; 1996). The conserved region is comprised of six charged amino acids at the extreme carboxy terminus coupled to 15-20 hydrophobic amino acids presumed to function as a transmembrane domain. Immediately adjacent to the transmembrane domain is a six amino acid sequence conserved in nearly all proteins ex-

specificity of antigen-antibody interaction at the level of individual amino acids. Proc. Natl. Acad. Sci. USA 82: 5131-5135; this "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten et al. (1986). Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow, M. et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle, F. J. et al., J. Gen. Virol. 66:2347-2354 (1985).

Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. See, for instance, Geysen et al., supra. Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. et al. (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

Table 4 lists immunologically useful polypeptides identified by an algorithm which locates novel *Staphylococcus aureus* outermembrane proteins, as is described above. Also listed are epitopes or "antigenic regions" of each of the identified polypeptides. The antigenic regions, or epitopes, are delineated by two numbers x-y, where x is the number of the first amino acid in the open reading frame included within the epitope and y is the number of the last amino acid in the open reading frame included within the epitope. For example, the first epitope in ORF 168-6 is comprised of amino acids 36 to 45 of SEQ ID NO:5,192, as is described in Table 4. The inventors have identified several epitopes for each of the antigenic polypeptides identified in Table 4. Accordingly, forming part of the present invention are polypeptides comprising an amino acid sequence of one or more antigenic regions identified in Table 4. The invention further provides polynucleotides encoding such polypeptides.

The present invention further includes isolated polypeptides, proteins and nucleic acid molecules which are substantially equivalent to those herein described. As used herein, substantially equivalent can refer both to nucleic acid and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between reference and subject sequences. For purposes of the present invention, sequences having equivalent biological activity, and equivalent expression characteristics are considered substantially equivalent. For purposes of determining equivalence, truncation of the mature sequence should be disregarded.

The invention further provides methods of obtaining homologs from other strains of *Staphylococcus aureus*, of the fragments of the *Staphylococcus aureus* genome of the present invention and homologs of the proteins encoded by the ORFs of the present invention. As used herein, a sequence or protein of *Staphylococcus aureus* is defined as a homolog of a fragment of the *Staphylococcus aureus* fragments or contigs or a protein encoded by one of the ORFs of the present invention, if it shares significant homology to one of the fragments of the *Staphylococcus aureus* genome of the present invention or a protein encoded by one of the ORFs of the present invention. Specifically, by using the sequence disclosed herein as a probe or as primers, and techniques such as PCR cloning and colony/plaque hybridization, one skilled in the art can obtain homologs.

As used herein, two nucleic acid molecules or proteins are said to "share significant homology" if the two contain regions which prossess greater than 85% sequence (amino acid or nucleic acid) homology. Preferred homologs in this regard are those with more than 90% homology. Especially preferred are those with 93% or more homology. Among especially preferred homologs those with 95% or more homology are particularly preferred. Very particularly preferred among these are those with 97% and even more particularly preferred among those are homologs with 99% or more homology. The most preferred homologs among these are those with 99.9% homology or more. It will be understood that, among measures of homology, identity is particularly preferred in this regard.

Region specific primers or probes derived from the nucleotide sequence provided in SEQ ID NOS:1-5,191 or from a nucleotide sequence at least 95%, particularly at least 99%, especially at least 99.5% identical to a sequence of SEQ ID NOS:1-5,191 can be used to prime DNA synthesis and PCR amplification, as well as to identify colonies containing cloned DNA encoding a homolog. Methods suitable to this aspect of the present invention are well known and have been described in great detail in many publications such as, for example, Innis *et al.*, PCR PROTOCOLS, Academic Press, San Diego, CA (1990)).

When using primers derived from SEQ ID NOS:1-5,191 or from a nucleotide sequence having an aforementioned identity to a sequence of SEQ ID NOS:1-5,191, one skilled in the art will recognize that by employing high stringency conditions (*e.g.*, annealing at 50-60°C in 6X SSPC and 50% formamide, and washing at 50- 65°C in 0.5X SSPC) only sequences which are greater than 75% homologous to the primer will be amplified. By employing lower stringency

quantitative determination of glucose in body fluids recently in biotechnology for analyzing syrups from starch and cellulose hydrosylates. This application is described in Owusu *et al., Biochem. et Biophysica. Acta.* 872: 83 (1986), for instance.

The main sweetener used in the world today is sugar which comes from sugar beets and sugar cane. In the field of industrial enzymes, the glucose isomerase process shows the largest expansion in the market today. Initially, soluble enzymes were used and later immobilized enzymes were developed (Krueger *et al.*, Biotechnology, The Textbook of Industrial Microbiology, Sinauer Associated Incorporated, Sunderland, Massachusetts (1990)). Today, the use of glucose- produced high fructose syrups is by far the largest industrial business using immobilized enzymes. A review of the industrial use of these enzymes is provided by Jorgensen, Starch 40:307 (1988).

Proteinases, such as alkaline serine proteinases, are used as detergent additives and thus represent one of the largest volumes of microbial enzymes used in the industrial sector. Because of their industrial importance, there is a large body of published and unpublished information regarding the use of these enzymes in industrial processes. (See Faultman *et al.*, Acid Proteases Structure Function and Biology, Tang, J., ed., Plenum Press, New York (1977) and Godfrey *et al.*, Industrial Enzymes, MacMillan Publishers, Surrey, UK (1983) and Hepner *et al.*, Report Industrial Enzymes by 1990, Hel Hepner & Associates, London (1986)).

Another class of commercially usable proteins of the present invention are the microbial lipases, described by, for instance, Macrae *et al.*, Philosophical Transactions of the Chiral Society of London 310:227 (1985) and Poserke, Journal of the American Oil Chemist Society 61:1758 (1984). A major use of lipases is in the fat and oil industry for the production of neutral glycerides using lipase catalyzed inter-esterification of readily available triglycerides. Application of lipases include the use as a detergent additive to facilitate the removal of fats from fabrics in the course of the washing procedures.

The use of enzymes, and in particular microbial enzymes, as catalyst for key steps in the synthesis of complex organic molecules is gaining popularity at a great rate. One area of great interest is the preparation of chiral intermediates. Preparation of chiral intermediates is of interest to a wide range of synthetic chemists particularly those scientists involved with the preparation of new pharmaceuticals, agrochemicals, fragrances and flavors. (See Davies *et al.*, Recent Advances in the Generation of Chiral Intermediates Using Enzymes, CRC Press, Boca Raton, Florida (1990)). The following reactions catalyzed by enzymes are of interest to organic chemists:hydrolysis of carboxylic acid esters, phosphate esters, amides and nitriles, esterification reactions, trans-esterification reactions, synthesis of amides, reduction of alkanones and oxoalkanates, oxidation of alcohols to carbonyl compounds, oxidation of sulfides to sulfoxides, and carbon bond forming reactions such as the aldol reaction.

When considering the use of an enzyme encoded by one of the ORFs of the present invention for biotransformation and organic synthesis it is sometimes necessary to consider the respective advantages and disadvantages of using a microorganism as opposed to an isolated enzyme. Pros and cons of using a whole cell system on the one hand or an isolated partially purified enzyme on the other hand, has been described in detail by Bud *et al.*, Chemistry in Britain (1987), p. 127.

Amino transferases, enzymes involved in the biosynthesis and metabolism of amino acids, are useful in the catalytic production of amino acids. The advantages of using microbial based enzyme systems is that the amino transferase enzymes catalyze the stereo- selective synthesis of only L-amino acids and generally possess uniformly high catalytic rates. A description of the use of amino transferases for amino acid production is provided by Roselle-David, *Methods of Enzymology* 136:479 (1987).

Another category of useful proteins encoded by the ORFs of the present invention include enzymes involved in nucleic acid synthesis, repair, and recombination. A variety of commercially important enzymes have previously been isolated from members of *Staphylococcus aureus*. These include Sau3A and Sau96I.

2. Generation of Antibodies

As described here, the proteins of the present invention, as well as homologs thereof, can be used in a variety procedures and methods known in the art which are currently applied to other proteins. The proteins of the present invention can further be used to generate an antibody which selectively binds the protein. Such antibodies can be either monoclonal or polyclonal antibodies, as well fragments of these antibodies, and humanized forms.

The invention further provides antibodies which selectively bind to one of the proteins of the present invention and hybridomas which produce these antibodies. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing polyclonal and monoclonal antibodies as well as hybridomas capable of producing the desired antibody are well known in the art (Campbell, A. M., MONOCLONAL ANTIBODY TECHNOLOGY: LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth *et al., J. Immunol. Methods* 35: 1-21 (1980), Kohler and Milstein, *Nature* 256: 495-497 (1975)), the trioma technique, the human B- cell hybridoma technique (Kozbor *et al., Immunology Today*

on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention.

Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers which comprises:(a) a first container comprising one of the Dfs, antigens or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following:wash reagents, reagents capable of detecting presence of a bound DF, antigen or antibody.

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, *etc.*), and containers which contain the reagents used to detect the bound antibody, antigen or DF.

Types of detection reagents include labelled nucleic acid probes, labelled secondary antibodies, or in the alternative, if the primary antibody is labelled, the enzymatic, or antibody binding reagents which are capable of reacting with the labelled antibody. One skilled in the art will readily recognize that the disclosed Dfs, antigens and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

4. Screening Assay for Binding Agents

Using the isolated proteins of the present invention, the present invention further provides methods of obtaining and identifying agents which bind to a protein encoded by one of the ORFs of the present invention or to one of the fragments and the *Staphylococcus aureus* fragment and contigs herein described.

In general, such methods comprise steps of:

(a) contacting an agent with an isolated protein encoded by one of the ORFs of the present invention, or an isolated fragment of the Staphylococcus aureus genome; and

(b) determining whether the agent binds to said protein or said fragment.

The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention.

Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby *et al.*, Application of Synthetic Peptides: Antisense Peptides," In Synthetic Peptides, A User's Guide, W. H. Freeman, NY (1992), pp. 289-307, and Kaspczak *et al.*, Biochemistry 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control.

One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix by binding to DNA or RNA. Such agents can be based on the classic phosphodiester, ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods usually contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al.*, Nucl. Acids Res. 6:3073 (1979); Cooney *et al.*, Science 241:456 (1988): and Dervan *et al.*, Science 251: 1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated

be measured at the same time. The composition of the present invention can be administered concurrently with, prior to, or following the administration of the other agent.

The agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to decrease the rate of growth (as defined above) of the target organism.

The administration of the agent(s) of the invention may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the agent(s) are provided in advance of any symptoms indicative of the organisms growth. The prophylactic administration of the agent(s) serves to prevent, attenuate, or decrease the rate of onset of any subsequent infection. When provided therapeutically, the agent(s) are provided at (or shortly after) the onset of an indication of infection. The therapeutic administration of the compound(s) serves to attenuate the pathological symptoms of the infection and to increase the rate of recovery.

The agents of the present invention are administered to a subject, such as a mammal, or a patient, in a pharmaceutically acceptable form and in a therapeutically effective concentration. A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

The agents of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby these materials, or their functional derivatives, are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, *e.g.*, human serum albumin, are described, for example, in REMINGTON'S PHARMACEUTICAL SCIENCES, 16th Ed., Osol, A., Ed., Mack Publishing, Easton PA (1980). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of one or more of the agents of the present invention, together with a suitable amount of carrier vehicle.

Additional pharmaceutical methods may be employed to control the duration of action. Control release preparations may be achieved through the use of polymers to complex or absorb one or more of the agents of the present invention. The controlled delivery may be effectuated by a variety of well known techniques, including formulation with macromolecules such as, for example, polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine, sulfate, adjusting the concentration of the macromolecules and the agent in the formulation, and by appropriate use of methods of incorporation, which can be manipulated to effectuate a desired time course of release. Another possible method to control the duration of action by controlled release preparations is to incorporate agents of the present invention into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization with, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in REMINGTON'S PHARMACEUTICAL SCIENCES (1980).

The invention further provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

In addition, the agents of the present invention may be employed in conjunction with other therapeutic compounds.

6. Shot-Gun Approach to Megabase DNA Sequencing

The present invention further demonstrates that a large sequence can be sequenced using a random shotgun approach. This procedure, described in detail in the examples that follow, has eliminated the up front cost of isolating and ordering overlapping or contiguous subclones prior to the start of the sequencing protocols.

Certain aspects of the present invention are described in greater detail in the examples that follow. The examples are provided by way of illustration. Other aspects and embodiments of the present invention are contemplated by the inventors, as will be clear to those of skill in the art from reading the present disclosure.

mercaptoethanol was added to the aliquot of cells to a final concentration of 25 mM. Cells were incubated on ice for 10 min. A 1 ul aliquot of the final ligation was added to the cells and incubated on ice for 30 min. The cells were heat pulsed for 30 sec. at 42° C and placed back on ice for 2 min. The outgrowth period in liquid culture was eliminated from this protocol in order to minimize the preferential growth of any given transformed cell. Instead the transformation mixture was plated directly on a nutrient rich SOB plate containing a 5 ml bottom layer of SOB agar (5% SOB agar: 20 g tryptone, 5 g yeast extract, 0.5 g NaCl, 1.5% Difco Agar per liter of media). The 5 ml bottom layer is supplemented with 0.4 ml of 50 mg/ml ampicillin per 100 ml SOB agar. The 15 ml top layer of SOB agar is supplemented with 1 ml X-Gal (2%), 1 ml $MgCl_2$ (1 M), and 1 ml $MgSO_4$/100 ml SOB agar. The 15 ml top layer was poured just prior to plating. Our titer was approximately 100 colonies/10 ul aliquot of transformation.

All colonies were picked for template preparation regardless of size. Thus, only clones lost due to "poison" DNA or deleterious gene products would be deleted from the library, resulting in a slight increase in gap number over that expected.

3. Random DNA Sequencing

High quality double stranded DNA plasmid templates were prepared using an alkaline lysis method developed in collaboration with 5Prime → 3Prime Inc. (Boulder, CO). Plasmid preparation was performed in a 96-well format for all stages of DNA preparation from bacterial growth through final DNA purification. Average template concentration was determined by running 25% of the samples on an agarose gel. DNA concentrations were not adjusted.

Templates were also prepared from a *Staphylococcus aureus* lambda genomic library. An unamplified library was constructed in Lambda DASH II vector (Stratagene). *Staphylococcus aureus* DNA (> 100 kb) was partially digested in a reaction mixture (200 ul) containing 50 ug DNA, 1X Sau3AI buffer, 20 units Sau3AI for 6 min. at 23 C. The digested DNA was phenol-extracted and centrifuges over a 10- 40% sucroce gradient. Fractions containing genomic DNA of 15-25 kb were recovered by precipitation . One ul of fragments was used with 1 ul of DASHII vector (Stratagene) in the recommended ligation reaction. One ul of the ligation mixture was used per packaging reaction following the recommended protocol with the Gigapack II XL Packaging Extract Phage were plated directly without amplification from the packaging mixture (after dilution with 500 ul of recommended SM buffer and chloroform treatment). Yield was about $2.5x10^9$ pfu/ul.

An amplified library was prepared from the primary packaging mixture according to the manufactureer's protocol. The amplified library is stored frozen in 7% dimethylsulfoxide. The phage titer is approximately $1x10^9$ pfu/ml.

Mini-liquid lysates (0.1ul) are prepared from randomly selected plaques and template is prepared by long range PCR. Samples are PCR amplified using modified T3 and T7 primers, and Elongase Supermix (LTI).

Sequencing reactions are carried out on plasmid templates using a combination of two workstations (BIOMEK 1000 and Hamilton Microlab 2200) and the Perkin-Elmer 9600 thermocycler with Applied Biosystems PRISM Ready Reaction Dye Primer Cycle Sequencing Kits for the M13 forward (M13-21) and the M13 reverse (M13RP1) primers. Dye terminator sequencing reactions are carried out on the lambda templates on a Perkin-Elmer 9600 Thermocycler using the Applied Biosystems Ready Reaction Dye Terminator Cycle Sequencing kits. Modified T7 and T3 primers are used to sequence the ends of the inserts from the Lambda DASH II library. Sequencing reactions are on a combination of AB 373 DNA Sequencers and ABI 377 DNA sequencers. All of the dye terminator sequencing reactions are analyzed using the 2X 9 hour module on the AB 377. Dye primer reactions are analyzed on a combination of ABI 373 and ABI 377 DNA sequencers. The overall sequencing success rate very approximately is about 85% for M13-21 and M13RP1 sequences and 65% for dye-terminator reactions. The average usable read length is 485 bp for M13-21 sequences, 445bp for M13RP1 sequences, and 375 bp for dye-terminator reactions.

4. Protocol for Automated Cycle Sequencing

The sequencing was carried out using Hamilton Microstation 2200, Perkin Elmer 9600 thermocyclers, ABI 373 and ABI 377 Automated DNA Sequencers. The Hamilton combines pre-aliquoted templates and reaction mixes consisting of deoxy- and dideoxynucleotides, the thermostable Taq DNA polymerase, fluorescently-labelled sequencing primers. and reaction buffer. Reaction mixes and templates were combined in the wells of a 96-well thermocycling plate and transferred to the Perkin Elmer 9600 thermocycler. Thirty consecutive cycles of linear amplification (i.e.., one primer synthesis) steps were performed including denaturation, annealing of primer and template, and extension; i.e., DNA synthesis. A heated lid with rubber gaskets on the thermocycling plate prevents evaporation without the need for an oil overlay.

Two sequencing protocols were used: one for dye-labelled primers and a second for dye-labelled dideoxy chain terminators. The shotgun sequencing involves use of four dye-labelled sequencing primers, one for each of the four terminator nucleotide. Each dye-primer was labelled with a different fluorescent dye, permitting the four individual reactions to be combined into one lane of the 373 or 377 DNA Sequencer for electrophoresis, detection, and base-

ORFs of at least 120 amino acids that did not match protein or nucleotide sequences in the databases at these levels are shown in Table 3.

ILLUSTRATIVE APPLICATIONS

1. Production of an Antibody to a *Staphylococcus aureus* Protein

Substantially pure protein or polypeptide is isolated from the transfected or transformed cells using any one of the methods known in the art. The protein can also be produced in a recombinant prokaryotic expression system, such as *E. coli*, or can by chemically synthesized. Concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the protein can then be prepared as follows.

2. Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., Nature 256:495 (1975) or modifications of the methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, E., Meth. Enzymol. 70:419 (1980), and modified methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. *et al.* Basic Methods in Molecular Biology Elsevier, New York. Section 21-2 (1989).

3. Polyclonal Antibody Production by Immunization

Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than other and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigenadministered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. *et al.*, J. Clin. Endocrinol. Metab. 33:988-991 (1971).

Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. *et al.*, Chap. 19 in:Handbook of Experimental Immunology, Wier, D., ed, Blackwell (1973). Plateau concentration of antibody is usually in the range of 0. 1 to 0. 2 mg/ml of serum (about 12M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in:Manual of Clinical Immunology, second edition, Rose and Friedman, eds., Amer. Soc. For Microbiology, Washington, D. C. (1980)

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi- quantitatively or qualitatively to identify the presence of antigen in a biological sample. In addition, they are useful in various animal models of Staphylococcal disease known to those of skill in the art as a means of evaluating the protein used to make the antibody as a potential vaccine target or as a means of evaluating the antibody as a potential immunothereapeutic reagent.

3. Preparation of PCR Primers and Amplification of DNA

Various fragments of the *Staphylococcus aureus* genome, such as those of Tables 1-3 and SEQ ID NOS:1-5,191 can be used, in accordance with the present invention, to prepare PCR primers for a variety of uses. The PCR primers are preferably at least 15 bases, and more preferably at least 18 bases in length. When selecting a primer sequence, it is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approxi-

GCTAATGGGA AATATGGAAT AAATGTGATT TGGTGATCAA CACAATATTG TAATACTGCC 2160

TCATTTTCGC GATGCAATAA ATTATATTCT AACTGTACAA CATCAACGTA ACCATCTTTA 2220

TTTGCTTCTT TAAGTTGATC TAATGTGAAA TTTGATACAC CAATTGCTTT AATCTTCCCT 2280

TGTTCCTTAA GCTCTTGTAA TGCTGCAACT GCTTGATCTT TCGGAGTGTT GTTATCCGGA 2340

AAATGAATAT AATATAAATC GATATAATCA GTTTGTAGAC GTTTCAAACT ATTCTCAACT 2400

TGTTGTTTTA AATATTCCGG TTGATTGTTC TGATGTACTT CTTGATTTTC ATCAAATTCA 2460

TGAGACCCTT TCGTAGCAAT TTTAATTTGC TCTCGCGGAT ATTCTTTAAC AACTTCTCCA 2520

ACCAATTCTT CTGATCGTTC TGGCCCATAA ATATATGCCG TATCTAATAA ATTAATACCA 2580

TGATTAATGG CTTGACGAAC AACATCTTTT CCTTGTTCTT CATCTAAGTT CGGATATAAA 2640

TTATGCCCAa CCTAtGCGTT CGTCCCAAGT GCGATTGGAA ACACTTCAAC ATCAGATTTA 2700

CCTAAGTTTA CAAATTGCTn CATTAGACCC AGCnCCTT 2738

(2) INFORMATION FOR SEQ ID NO: 87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9425 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

GATTAGATGA TATTTAACGA AAATTAaGrT GmAATACTtG AATGTArGAa GTCTGATGTC 60

GAAAATAGCT ATTAAAATAG AGTAGACGTA ATGtAAATGA AAGCACCTAA AATAGAAAAA 120

TTTCAAAAAT AGCGTAATTA TTATAATAAA TAGACTGCCA ATAAAATGCA ATTTTTCACT 180

TATAACATTC TTCAAAAAAT AATAGCAAAA TTATGTAAAA AATATCTTGT CATGGCAAGA 240

TTGGCTGTGC TATAATCTAT CTTGTGCTTA AGAACGGCTC CTTGGTCAAG CGGTTAAGAC 300

ACCGCCCTTT CACGGCGGTA ACACGGGTTC GAGTCCCGTA GGAGTCACCA TTTTTTAGGT 360

CTCGTAGTGT AGCGGTTAAC ACGCCTGCCT GTCACGCAGG AGATCGCGGG TTCGATTCCC 420

GTCGAGACCG TACAAATGCC TATCCAAGAG GATAGGCATT TTTTTGCGTT TAATATTATA 480

TTAATAAAAG ATATATGGAC GAATGATAAT CATATTGATT TATCTGTTCG TCCATTTTCT 540

TTAAAATGTA TGAACCTCAA GTAACTTAGT GGTTGGATAT GAAAGATAAA CGTAGACAAT 600

AAAATCTTTA TTAGACGTAC AAACATATGC TACTGTCAAC ATATTTCTTC GTTGTGATAT 660

GCCACCAGTC CTCCATAACA TCAATTGTTA AAGTAACGAA TAACGAATAA TGATATTTAT 720

GTGATGGTAT TATTGCAACA GACCGCCGTG GACGTATTCG TATCGTCAAT GATATGGCAC 2640

TCAAGATGCT TGGTATGGCG AAAGAAGACA TCATCGGATA TTACATGTTA AGTGTATTAA 2700

GTCTTGAAGA TGAATTTAAA CTGGAAGAAA TTCAAGAGAA TAATGATAGT TTCTTATTAG 2760

ATTTAAATGA AGAAGAAGGT CTAATCGCAC GTGTTAACTT TAGTACGATT GTGCAGGAAA 2820

CAGGATTTGT AACTGGTTAT ATCGCTGTGT TACATGACGT AACTGAACAA CAACAAGTTG 2880

AACGTGAGCG TCGTGAATTT GTTGCCAATG TATCACATGA GTTACGTACA CCTTTAACTT 2940

CTATGAATAG TTACATTGAA GCACTTGAAG AAGGTGCATG GAAAGATGAG GAACTTGCGC 3000

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TGCTACAGTT ATCTAAAATG GATAATGAGT CTGATCAAAT CAACAAAGAA ATTATCGACT 3120

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(2) INFORMATION FOR SEQ ID NO: 2975:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 361 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2975:

GAGATCGCGG GTTCGATTCC CGTCGAGACC GTACAAATGC CTATCCAAGA GATAGTTTTG 60

TnTTGCGTTT AATATTATAT TAATAAAAGA TATATGGACG AATGATAATC ATATTGATTT 120

ATCTGTTCGT CCATTTTCTT TAAAATGTAT GAACCTCAAG TAACTTAGTG GTTGGATATG 180

AAAGATAAAC GTnGACAATA AAATCTTTAT TAGACGTACA AACATATGCT ACTGTCAACA 240

TATTTCTTCG TTGTGATATG CCACCAGTCC TCCATAACAT CAATTGTTAA AGTAACGAAT 300

AACGAATAAT GATATTTnAT TTCTGAGCCA TGACGTGCCA CTAGAAGTTG CCCATTATCC 360

T 361

(2) INFORMATION FOR SEQ ID NO: 2976:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 356 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2976:

TGCTGCTTCT GAAGCGGAGT AATTGTGTAT ATCAAATGTC GCTTTTTGAT CAGGAACATG 60

ATGTAAAGGT GTATATGTTT CACCTGTTTG CTCATCATGT AACACGGCAT GCCTATGACT 120

GAATGTACCA CGTTCACTAT CTTGACCAGT TAAGCGAATC GGTGTACCAT CTTGTAAAAT 180

TGTCGCAAAT GCAAGTnGTT CTGCTTGTGC CCAATCAACT AAACCATCTT CTTTATTAAA 240

CGGCTCATGA CGCTTCTCAA GAACTTTGTT TAACTTTTTn CAAAATGTTA AAGCCATCCG 300

GATATGTTAA CAATGCATCA TTATTTCTTT CAAGTGATCC AAAAGTAAAG GnTTGT 356

(2) INFORMATION FOR SEQ ID NO: 2977:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 371 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

Arg Asp Gly Met Glu Val Cys Arg Glu Val Arg Lys Lys Tyr Glu Met
65 70 75 80

Pro Ile Ile Met Leu Thr Ala Lys Asp Ser Glu Ile Asp Lys Val Leu
85 90 95

Gly Leu Glu Leu Gly Ala Asp Asp Tyr Val Thr Lys Pro Phe Ser Thr
100 105 110

Arg Glu Leu Ile Ala Arg Val Lys Ala Asn Leu Arg Arg His Tyr Ser
115 120 125

Gln Pro Ala Gln Asp Thr Gly Asn Val Thr Asn Glu Ile Thr Ile Lys
130 135 140

Asp Ile Val Ile Tyr Pro Asp Ala Tyr Ser Ile Lys Lys Arg Gly Glu
145 150 155 160

Asp Ile Glu Leu Thr His Arg Glu Phe Glu Leu Phe His Tyr Leu Ser
165 170 175

Lys His Met Gly Gln Val Met Thr Arg Glu His Leu Leu Gln Thr Val
180 185 190

Trp Gly Tyr Asp Tyr Phe Gly Asp Val Arg Thr Val Asp Val Thr Ile
195 200 205

Arg Arg Leu Arg Glu Lys Ile Glu Asp Asp Pro Ser His Pro Glu Tyr
210 215 220

Ile Val Thr Arg Arg Gly Val Gly Tyr Phe Leu Gln Gln His Glu
225 230 235

(2) INFORMATION FOR SEQ ID NO:5240:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5240:

Xaa Leu Ser Thr Val Ile Gly Ala Xaa Leu Phe Phe Lys Ser Ser Val
1 5 10 15

Ser Leu Val Phe Lys Met Val Lys Lys Phe Arg Xaa Gly Val Ile Ser
20 25 30

Val Asn Asp Val Met Phe Ser Ser Ser Ile Met Tyr Arg Ile Lys Lys
35 40 45

Asn Ala Phe Ser Leu Thr Val Met Ala Ile Ile Ser Ala Ile Thr Val
50 55 60

His Lys Ala
1025

(2) INFORMATION FOR SEQ ID NO:5255:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5255:

```
Gly Glu Lys Cys Met Phe Leu Ala Trp Asn Glu Ile Arg Arg Asn Lys
1               5                  10                  15

Leu Lys Phe Gly Leu Ile Ile Gly Val Leu Thr Met Ile Ser Tyr Leu
            20                  25                  30

Leu Phe Leu Leu Ser Gly Leu Ala Asn Gly Leu Ile Asn Met Asn Lys
        35                  40                  45

Glu Gly Ile Asp Lys Trp Gln Ala Asp Ala Ile Val Leu Asn Lys Asp
    50                  55                  60

Ala Asn Gln Thr Val Gln Gln Ser Val Phe Asn Lys Lys Asp Ile Glu
65                  70                  75                  80

Asn Lys Tyr Lys Lys Gln Ala Thr Leu Lys Gln Thr Gly Glu Ile Val
                85                  90                  95

Ser Asn Gly His Gln Lys Asp Asn Val Leu Val Phe Gly Val Glu Lys
            100                 105                 110

Ser Ser Phe Leu Val Pro Ser Leu Ile Glu Gly His Lys Ala Thr Lys
        115                 120                 125

Asp Asn Glu Val Leu Ala Asp Glu Thr Leu Lys Asn Lys Gly Leu Lys
    130                 135                 140

Leu Gly Asp Thr Leu Ser Leu Ser Xaa Xaa Arg
145                 150                 155
```

Claims

1. Computer readable medium having recorded thereon a nucleotide sequence of the Staphylococcus aureus genome as depicted in SEQ ID NOS:1-5,191, a representative fragment thereof or a nucleotide sequence at least 95 % identical to a nucleotide sequence depicted in SEQ ID NOS:1-5,191.

2. Computer readable medium having recorded thereon any one of the fragments of SEQ ID NOS:1-5,191 depicted in Tables 2 and 3 or a degenerate variant thereof.

of claim 12.

17. A method for regulating the expression of a nucleic acid molecule comprising the step of covalently attaching to said nucleic acid molecule a nucleic acid molecule consisting of the nucleotide sequence from about 30 to 300 bases 5' to any one of the fragments of the Staphylococcus aureus genome depicted in Seq ID Nos:1-5,191 and Tables 2 and 3 or a nucleotide sequence at least 95% identical to such a nucleotide sequence or a degenerate variant of any of the aforementioned sequences.

18. A nucleic acid molecule being a homolog of any of the fragments of the Staphylococcus aureus genome of SEQ ID NOS:1-5,191 and Tables 2 and 3, wherein said nucleic acid molecule is produced by a process comprising the steps of:

(a) screening a genomic DNA library using as a probe a target sequence defined by any of SEQ ID NOS: 1-5,191 and Tables 2 and 3, including fragments thereof;
(b) identifying members of said library which contain sequences that hybridize to said target sequence;
(c) isolating the nucleic acid molecules from said members identified in step (b).

19. A DNA molecule being a homolog of any one of the fragments of the Staphylococcus aureus genome of SEQ ID NOS:1-5,191 and Tables 2 and 3, wherein said nucleic acid molecule is produced by a process comprising the steps of:

(a) isolating mRNA, DNA, or cDNA produced from an organism;
(b) amplifying nucleic acid molecules whose nucleotide sequence is homologous to amplification primers derived from said fragment of said *Staphylococcus aureus* genome to prime said amplification;
(c) isolating said amplified sequences produced in step (b).

20. A polypeptide encoded by a fragment of claim 8.

21. An antibody which selectively binds to any one of the polypeptides of claim 20.

22. A kit for analyzing samples for the presence of polynucleotides derived from Staphylococcus aureus, comprising at least one polynucleotide containing a nucleotide sequence of any one of the fragments SEQ ID NOS:1-5,191 depicted in Tables 2 and 3 or a nucleotide sequence at least 95% identical thereto or a degenerate variant of any of the aforementioned sequences, that will hybridize to a staphylococcus aureus polynucleotide under stringent hybridization conditions, and a suitable container.

23. A Staphylococcus aureus polypeptide comprising an amino acid sequence identical to an amino acid sequence selected from the group consisting of SEQ ID NOS:5,192 to 5,255 or comprising an amino acid sequence having at least 95% identity to such a sequence.

24. A Staphylococcus aureus polypeptide antigen comprising at least one epitope derived from a Staphylococcus aureus polypeptide selected from the group consisting of SEQ ID NOS:5,192 to 5,255.

25. A polypeptide comprising at least one epitope encoded by a Staphylococcus aureus amino acid sequence selected from the group consisting of the epitopic sequences listed in Table 4.

26. The polypeptide of claim 24 or 26, wherein said polypeptide is fixed to a solid phase.

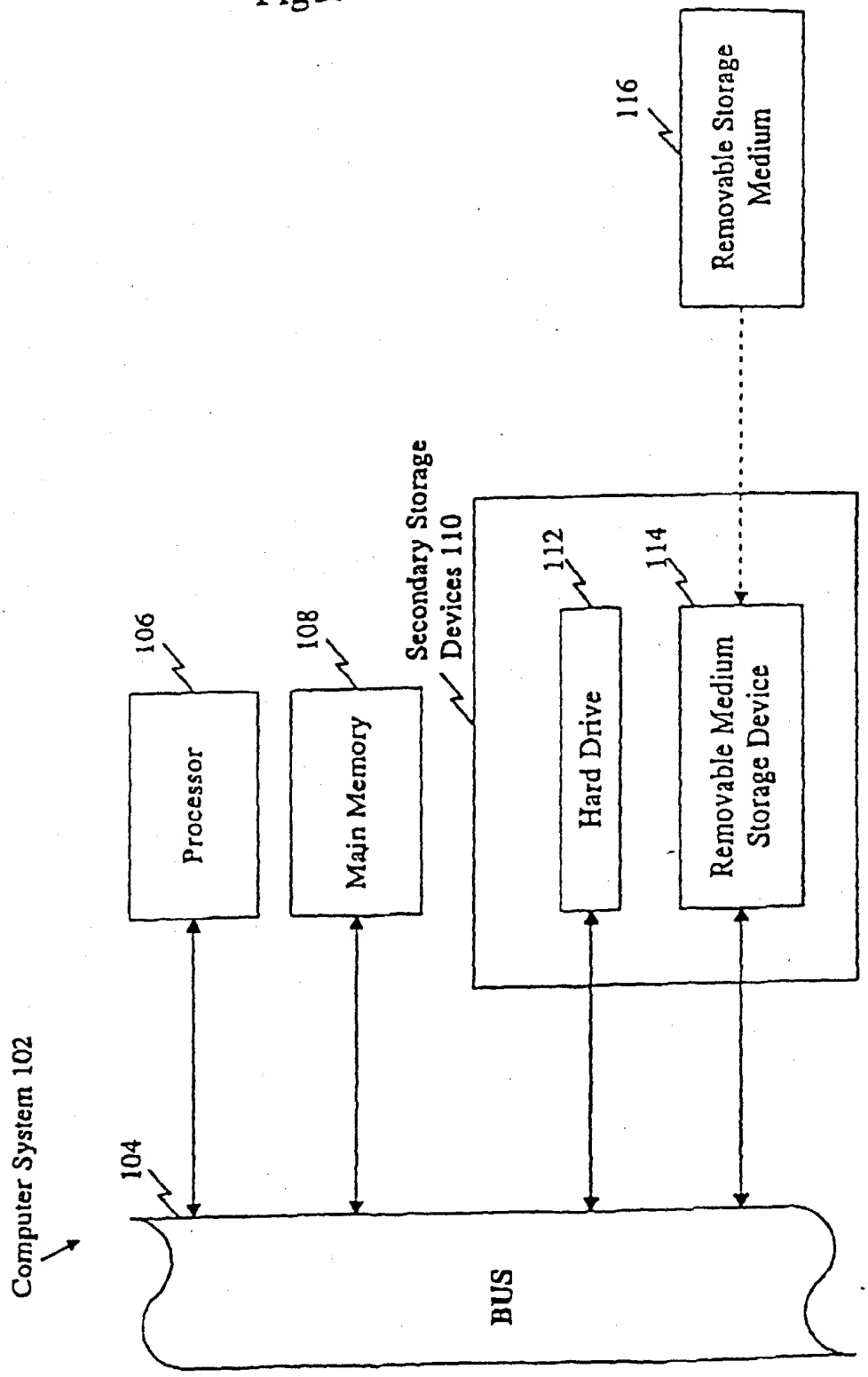
27. A diagnostic kit for detecting Staphylococcus aureus infection comprising

(a) an isolated polypeptide antigen of claim 24, and
(b) means for detecting the binding of an antibody contained in a biological fluid to said antigen.

28. A vaccine composition comprising a polypeptide of claim 24 present in a pharmaceutically acceptable carrier.

29. A method of vaccinating an individual against Staphylococcus aureus infection comprising, administering to an individual the vaccine composition of claim 28.

Figure 1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Main Memory
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Hard Drive
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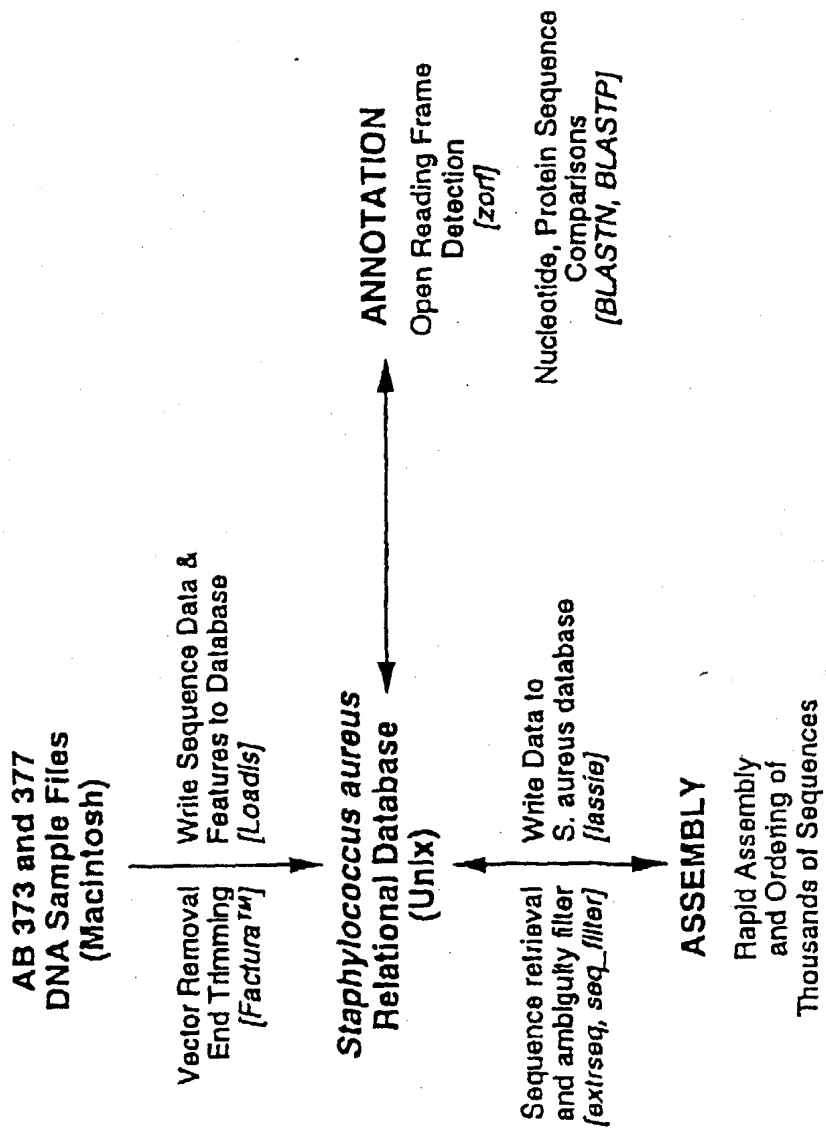
Removable Storage Medium

Figure 2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